



Microscopic visualization of lipid- and protein-mediated gas permeation across biological membranes at an atomic level

Paween Mahinthichaichan¹, Rossana Occipinti², Fraser J. Moss¹, Dengke Wang¹, Daniela Calvetti³, Erkki Somersalo³, Walter F. Boron², Emad Tajkhorshid²

1) Department of Biochemistry and Beckman Institute for Advanced Science & Technology, University of Illinois at Urbana-Champaign, Urbana, IL; 2) Department of Physiology & Biophysics, Case Western Reserve University, Cleveland, OH; 3) Department of Mathematics, Applied Mathematics & Statistics, Case Western Reserve University, Cleveland, OH.

Biological functions of gases

$O_2 \rightarrow$ Bioenergetics $CO_2 \rightarrow$ acid-based homeostasis
 $NO \rightarrow$ Cell signaling $CO_2 \rightarrow$ Biomass in plants

Extend to which membrane channels contribute to gas transport

Cellular membranes: resistant to O_2 and CO_2

Apical membrane of gastric parietal chief cells

S.J. Waisbren,..., W.F. Boron. Nature 1994

Fiber-cell plasma membrane of eye lenses

J. Widonska,..., W.K. Subczynski. BBA 2007

Abundance of protein channels, such as aquaporins and Rh glycoproteins, in erythrocyte membranes

- Inhibitor DIDS reduced CO_2 permeability by >10 folds.

R.E. Forster,..., M. Wunder. PNAS 1998

- Aquaporin-1 (AQP1) increased water and CO_2 permeability which were abolished by $HgCl_2$ or pCMBS (Hg derivative).

G.V. Ramesh Prasad,..., M.L. Zeidel. JBC 1998
V. Endeward,..., G. Gros. FASEB 2006

- Knockout RhAG resulted in a 2-fold decrease in permeability

V. Endeward,..., G. Gros. FASEB 2008

Chemically synthetic membranes: triblock copolymer

- Insertion of aquaporins increased CO_2 influx by 10-20 folds.

N. Uehlein,..., R. Kaldenholz. Sci. Reports 2012

- Adding cholesterol reduced CO_2 fluxes.

L. Kai and R. Kaldenholz. Sci. Reports 2014

Molecular dynamics (MD) simulation provides:

Time-averaged information of structure and dynamics of proteins and lipids at atomic resolution

Thermodynamics and kinetics associated with the transport of molecules

Microscopic diffusive permeability coefficient, p_d :

molar flow (J_n) of gas transport across a compartment (e.g. lipid membrane, protein channels) normalized by the cross-sectional area (A) of that compartment and the gas (e.g. CO_2 , O_2) concentration (C_b),

$$p_d = \frac{J_n}{C_b \langle A \rangle} = \frac{\langle n_p \rangle}{C_b \langle A \rangle (N_A \Delta t)}$$

n_p : number of gas molecules crossing a region at Δt

A : horizontal area of protein lumen or membrane patch

$p_d \propto K$ (partitioning coefficient or solubility constant of a gas species)

$K = e^{-\Delta G/RT}$ (ΔG : the partition free energy of the gas)

$\propto D$ (diffusion coefficient of the gas)

$\propto 1/L$ (pathway's length)

MD simulation preparations

POPC membrane-embedded AQP complexes for: AQP1 from PDB 1J4N, AQP5 from PDB 3GD8, and AQP7 (unpublished structure)

CHARMM-GUI to prepare membrane lipid patches of POPC, CHL and PSM (initial xy dimension of $100 \times 100 \text{ \AA}^2$)

Equilibrated for 25 to 100 ns before flooding simulations of CO_2

CHARMM36 FF and NAMD2 for simulations at 310 K and 1 atm

Gas permeability through membrane channels: CO_2 diffusion through aquaporins (AQPs)

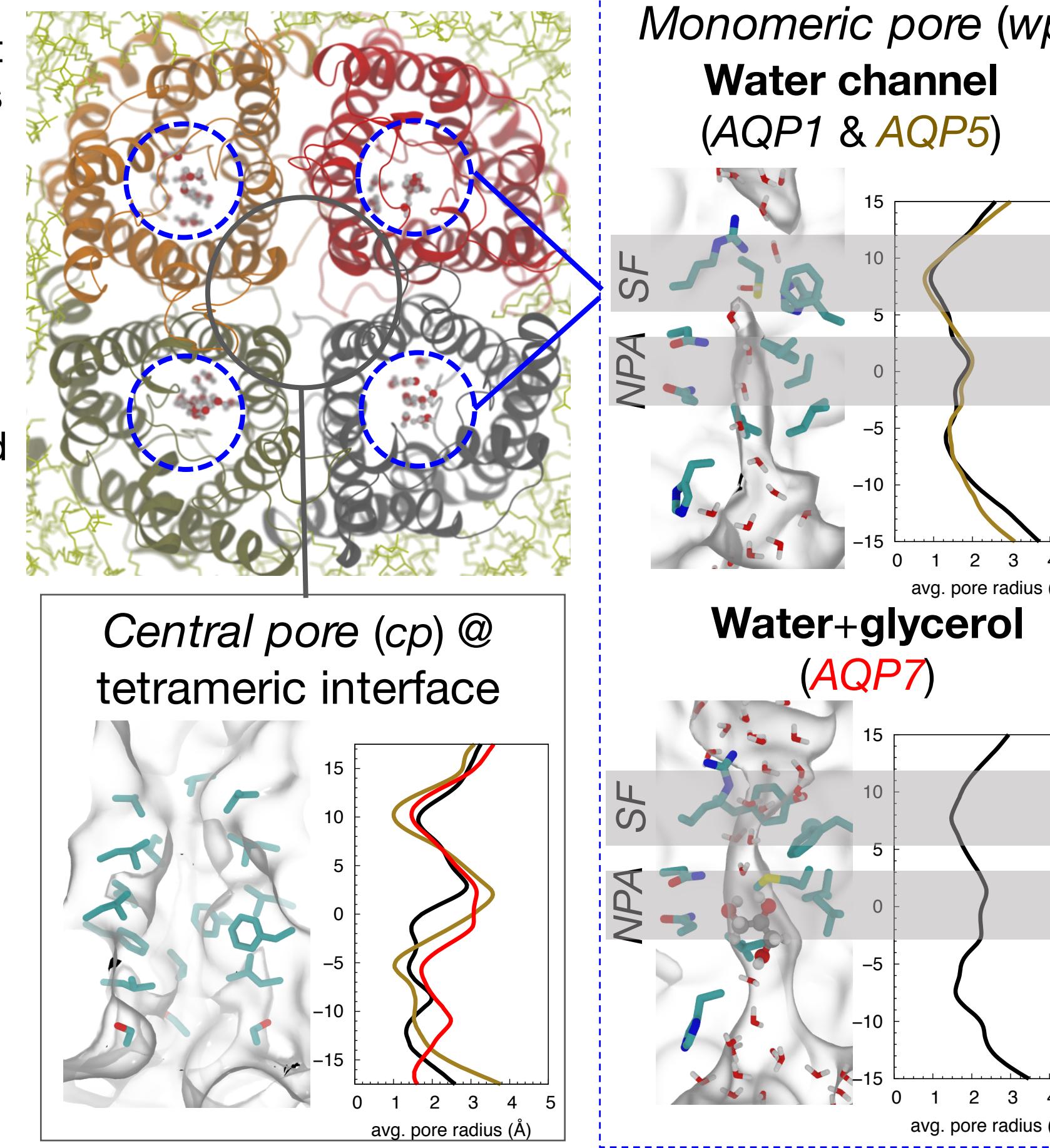
AQP1, AQP5, AQP7 homotetramers

AQPs are ones of the most abundant membrane channels in living organisms.

- 13 species in human

AQP1 and AQP5 function as water channels.

AQP7 permeates water and glycerol molecules.

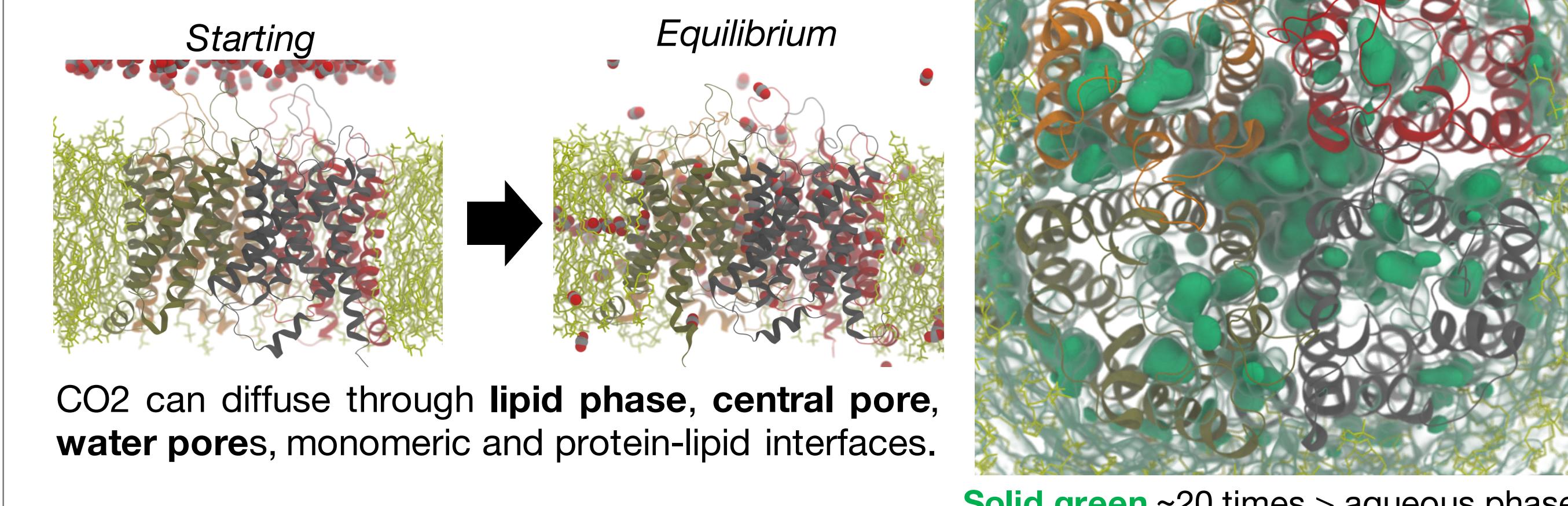


Flooding simulations of 125 CO_2 molecules in the AQPs embedded in POPC membrane lipids

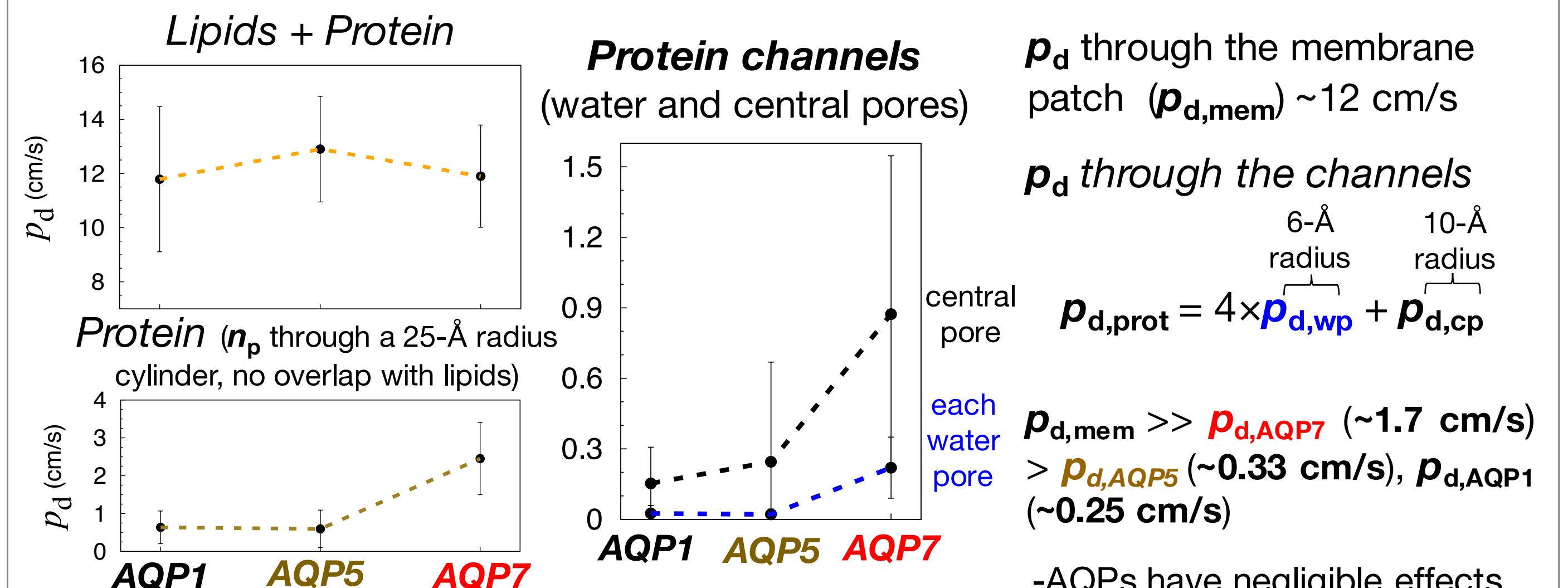
Simulation times: 750-1,000 ns long

Initial bulk $[CO_2]$ (C_b) ~400 mM → Final ~100 mM

Lipid/Protein ratio based on area ~ 2 to 1

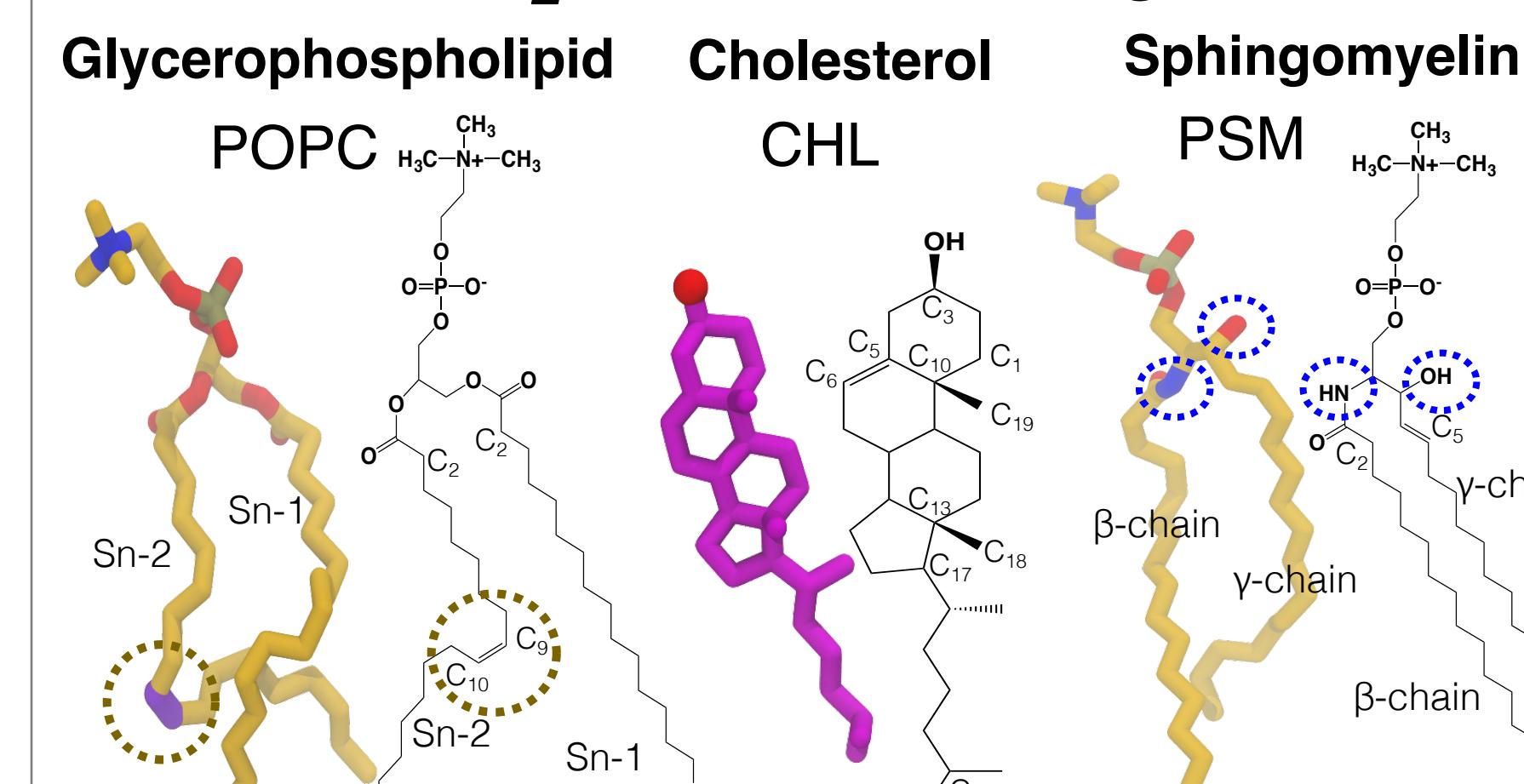


Calculated p_d values of CO_2 through the entire membrane patch, protein domain and individual pores (wp & cp)



Membrane lipid composition on gas permeability

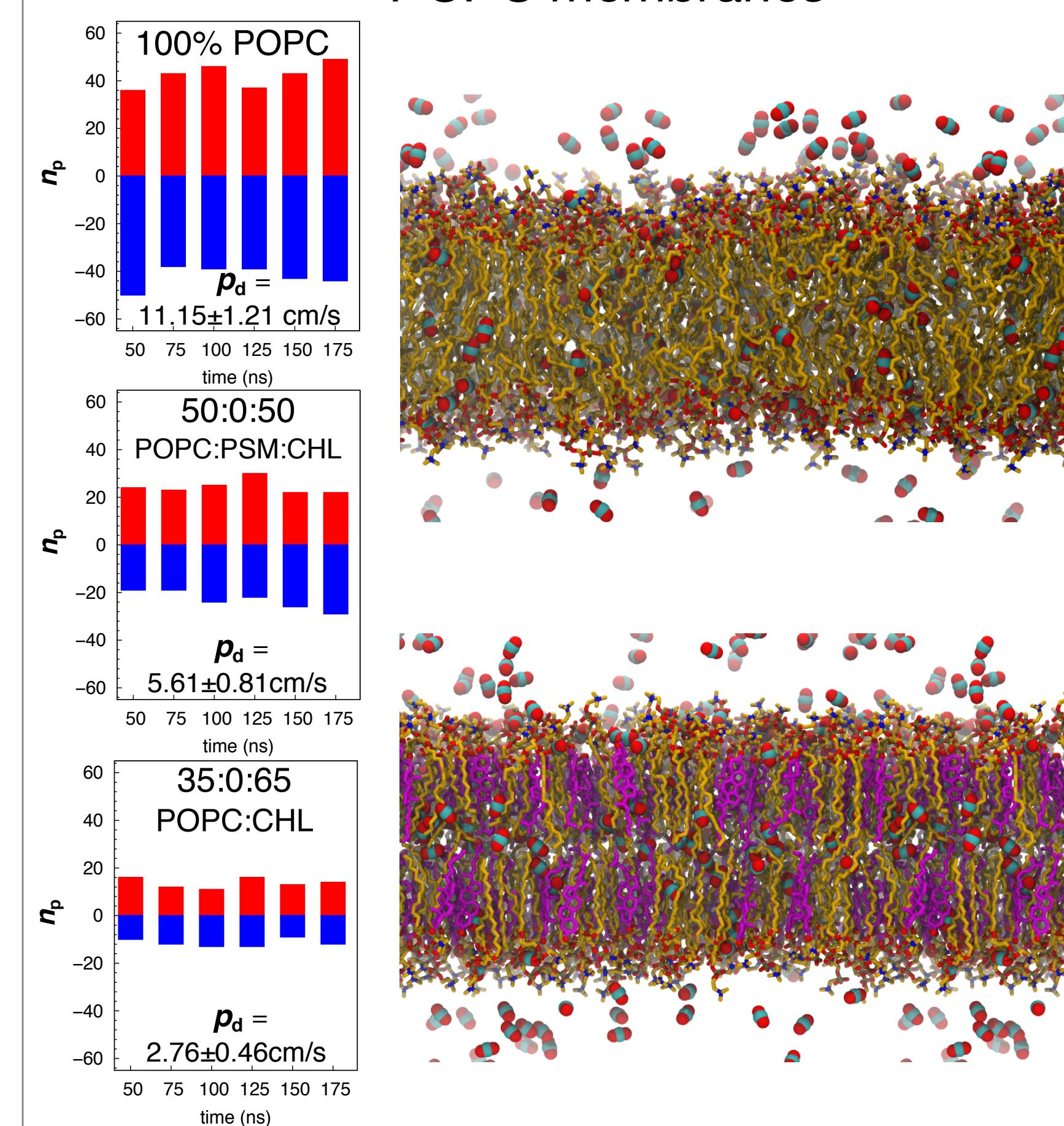
CO_2 diffusion through various lipid membranes



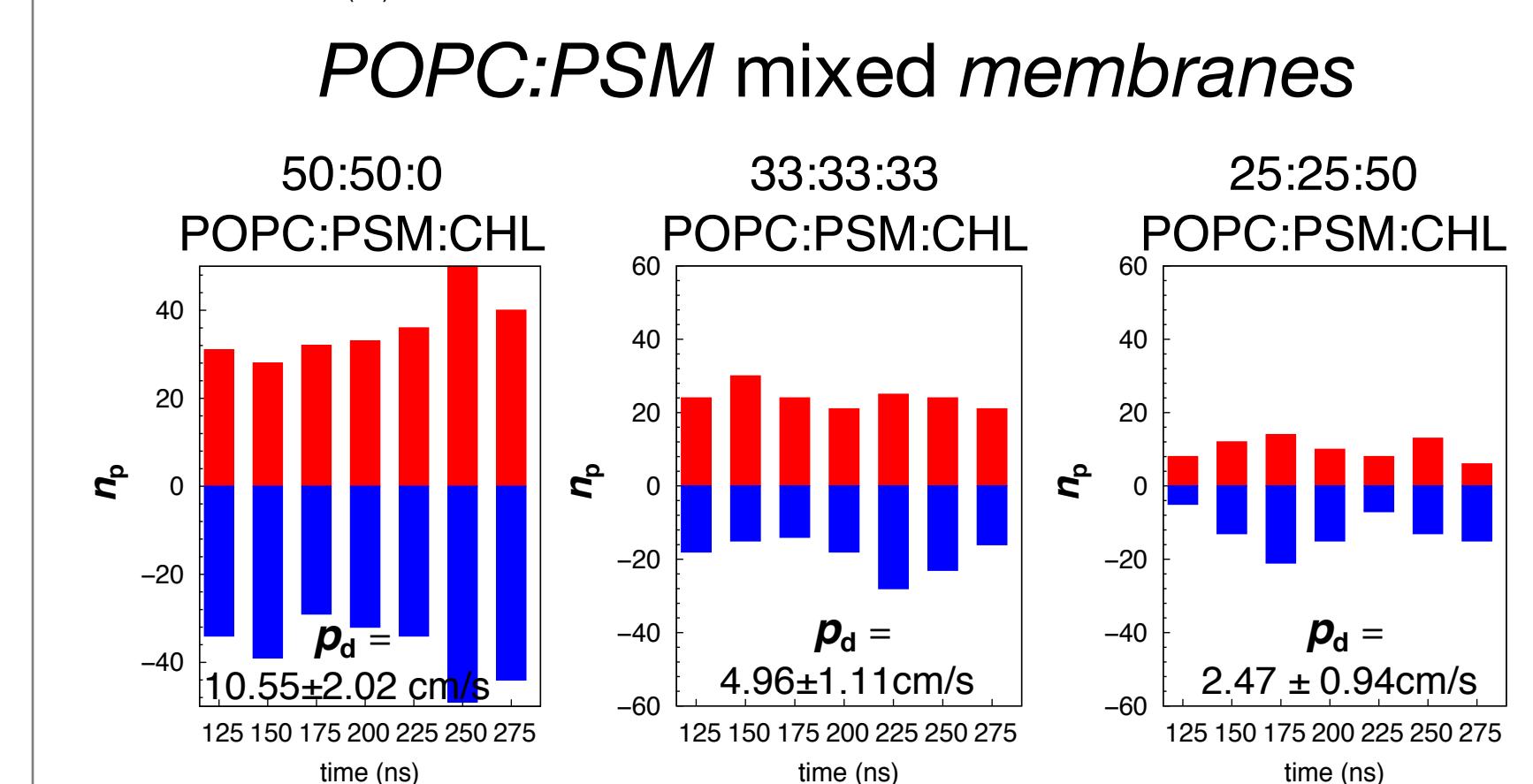
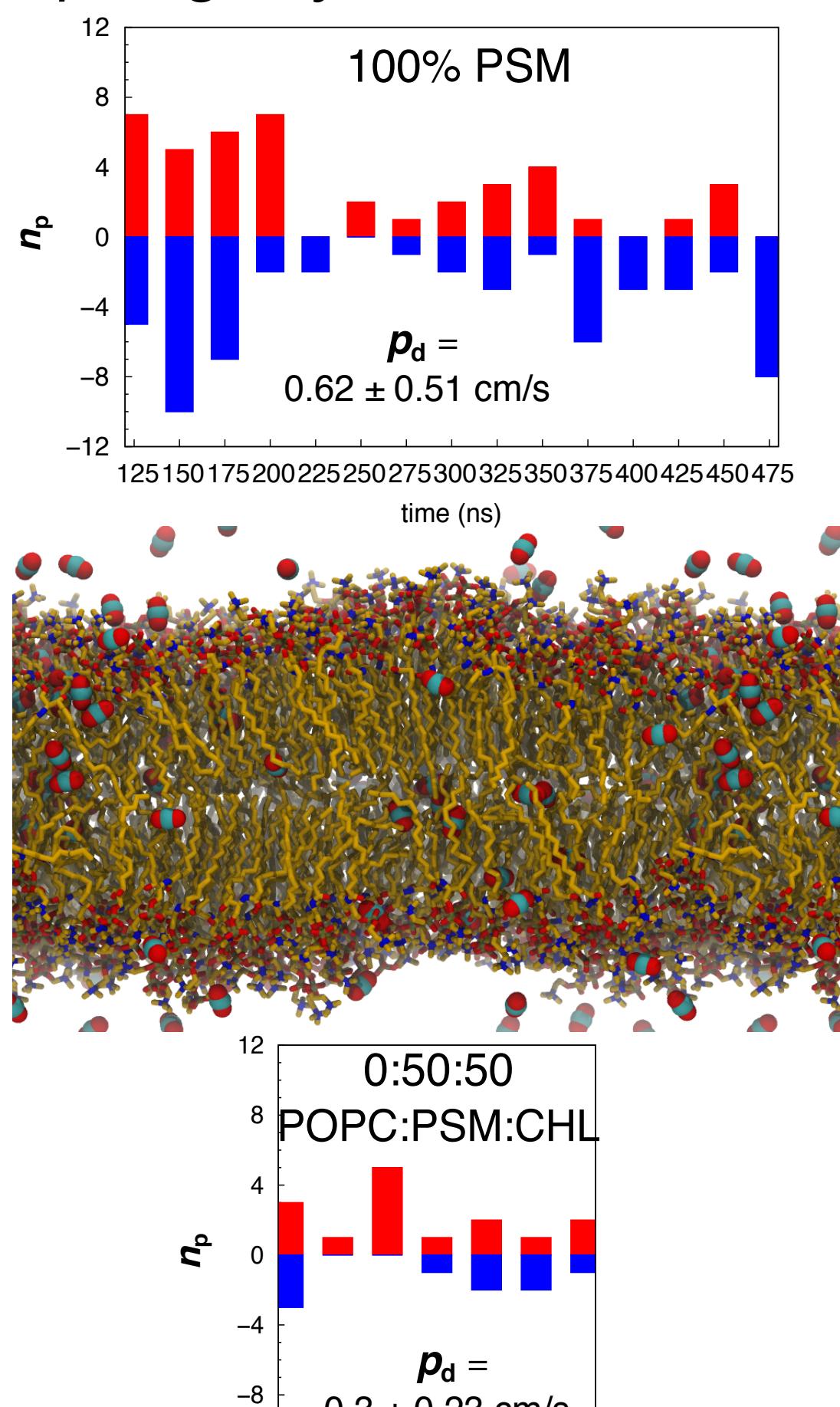
Equilibrated systems

POPC:PSM:CHL	Total time (ns)	$\langle A_{mem} \rangle (\text{\AA}^2)$	$\langle C_{bulk} \rangle (M)$
100:0:0	225	9,500	0.133
50:0:50	225	8,000	0.176
35:0:65	225	9,000	0.169
0:100:0	500	9,225	0.189
0:50:50	325	8,350	0.229
50:50:0	325	9,460	0.122
33:33:33	325	8,160	0.177
25:25:50	325	8,200	0.188

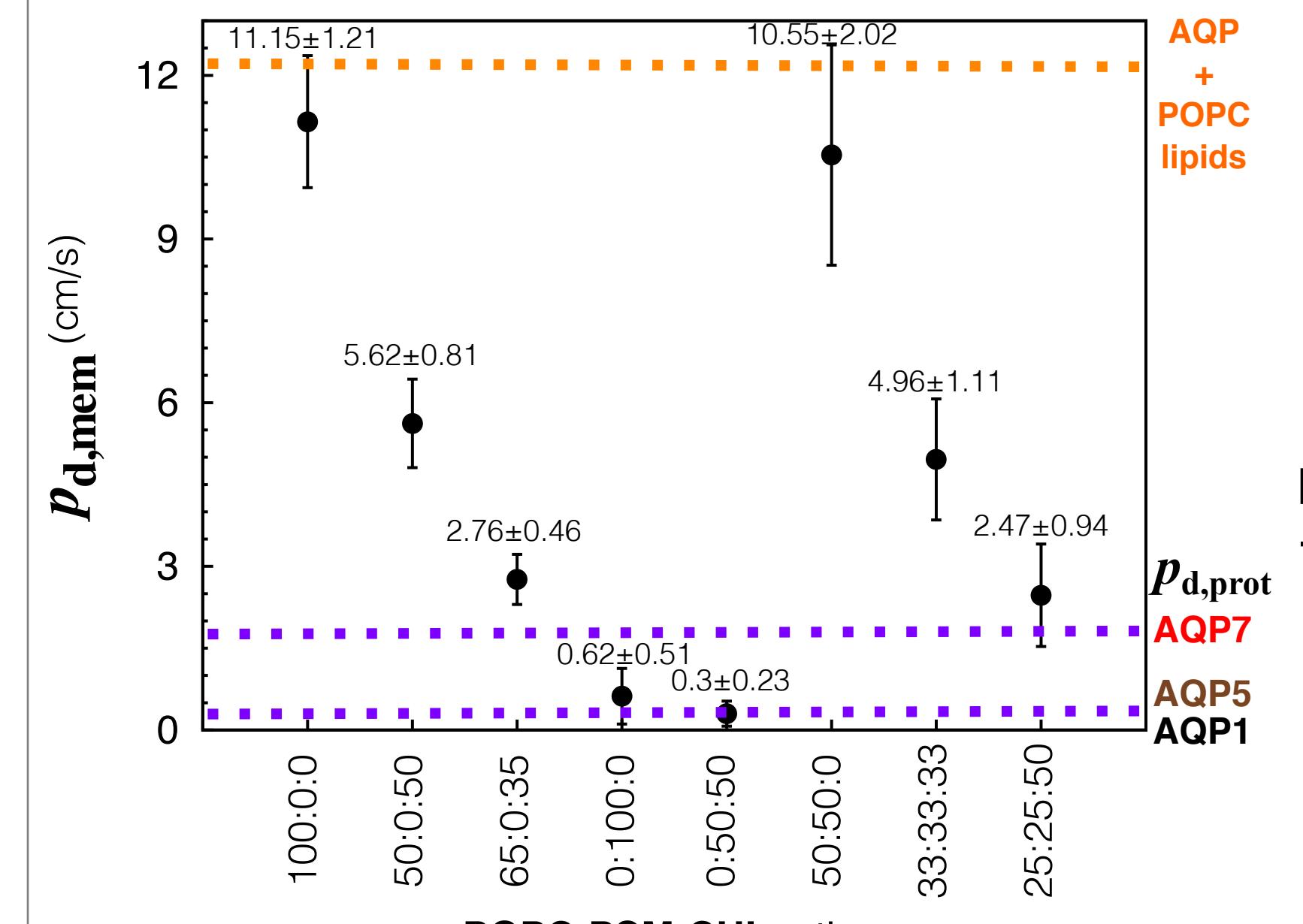
POPC membranes



Sphingomyelin membranes



Diffusion rates of CO_2 in lipids versus AQP channels



CO_2 diffuses more freely in 100% POPC and 50:50 POPC:PSM membranes.

p_d,mem with AQP embedded ~ $p_d,POPc$ >> $p_d,AQP7, AQP5, AQP1$

More cholesterol, more sphingomyelin → Lower permeability

The roles of AQPs become obvious when reconstituted in membranes with 100% PSM or 50:50 PSM:CHL, where $p_d,prot \geq p_d,mem$.